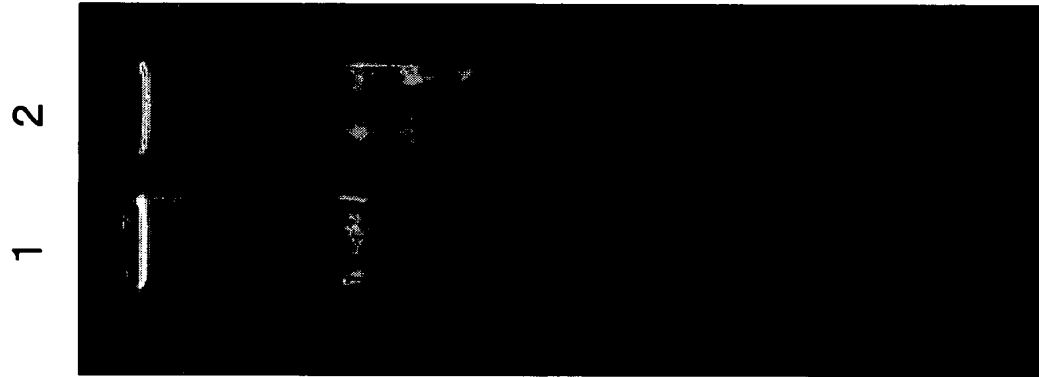


# Material 1

Best Available Copy



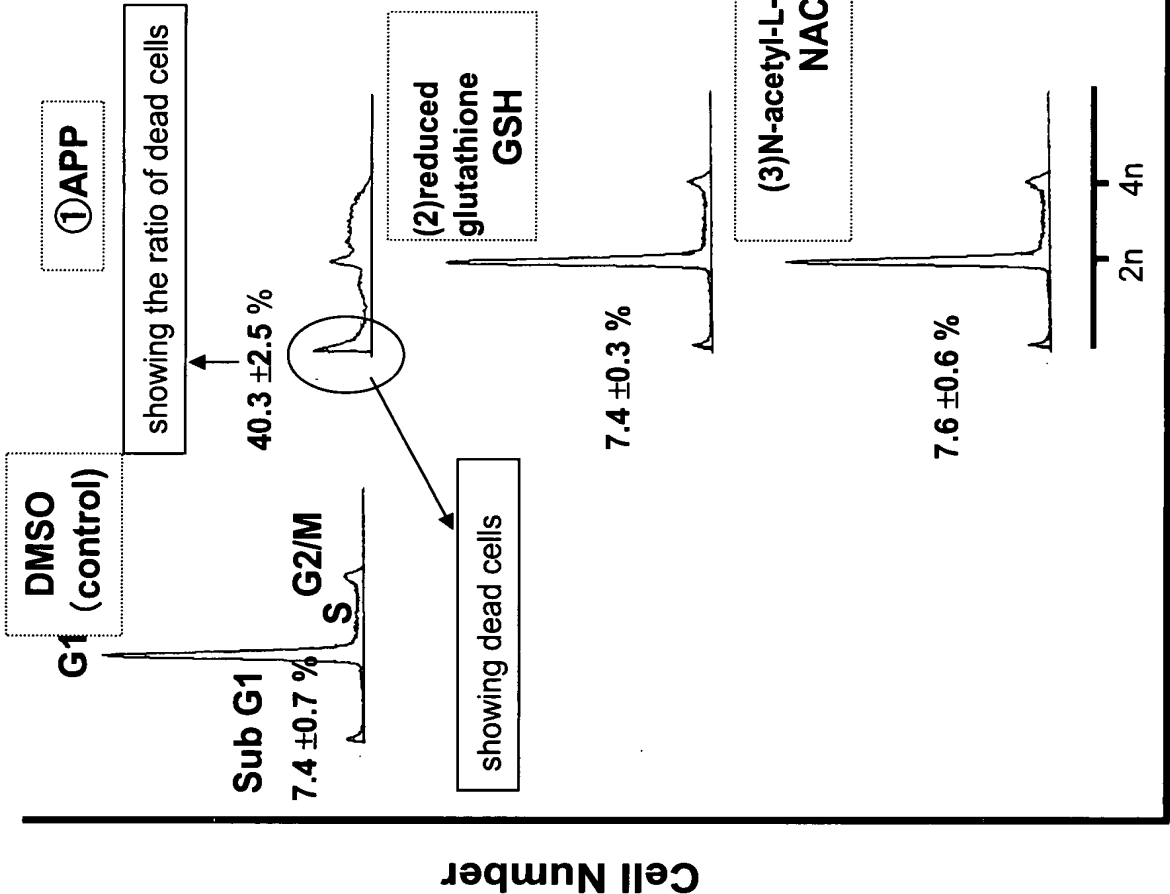
Lane 1: Control(DMSO)  
Lane 2: APP(1.4mM)

Cell strain: HaCaT  
Time: 48hr

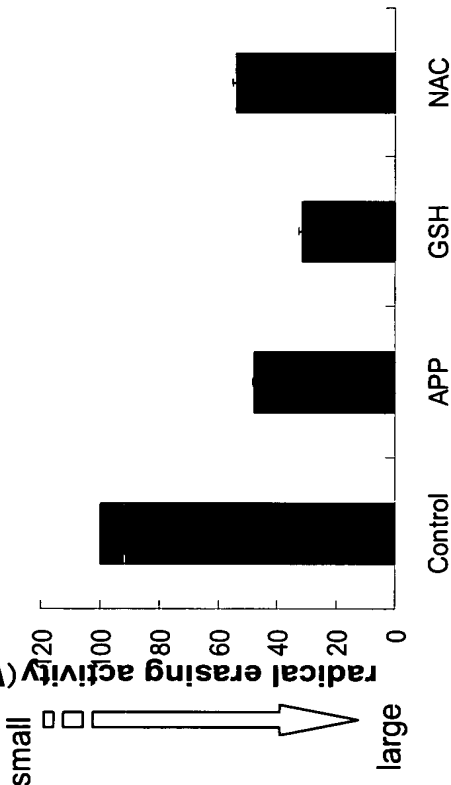
Material 2

Comparison between APP and antioxidant material

A. cell killing effect



B. antioxidant activity (comparison of DPPH radical erasing activity)



Sub G1, G1, S and GS/M in Fig. A show the cell cycles of a cancer cell. (1) shows that APP is added to a cancer cell, (2) shows that GSH is added to a cancer cell, and (3) shows that NAC is added to a cancer cell. DMSO is used as a control and this graph shows a normal cell cycle. Since (2) and (3) are similar to the graph of DMSO, it is considered that GSH and NAC have no influence upon the cancer cell. However, it is understood from (1) that APP has an influence upon the cell cycle of the cancer cell and disturbs the cell cycle.

Details are shown in Material 3.



### Material 3

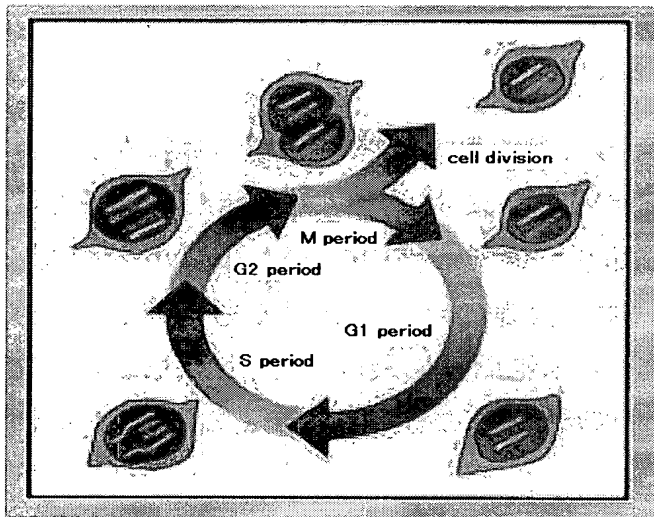
#### A. cell killing effect

##### 1) In re method of conducting experiments

What is important for the evaluation of an antitumor effect (*in vitro*) is whether a substance has a cell killing effect or not. Material 2-A shows the results of investigation into this effect. Additional information on this material is given below.

The feature of a cancer cell will be first described. The cancer cell has proliferating ability which cannot be controlled and immortality. That is, one cancer cell is divided into two which are further divided into four without requiring fertilization. Fig. 1 shows this. This is called "cell cycle".

Fig. 1 proliferation of cell



#### Explanation of the figure

G1 period: sister cell  
(so-called "ordinary cell")

S period: DNA synthesizing  
period

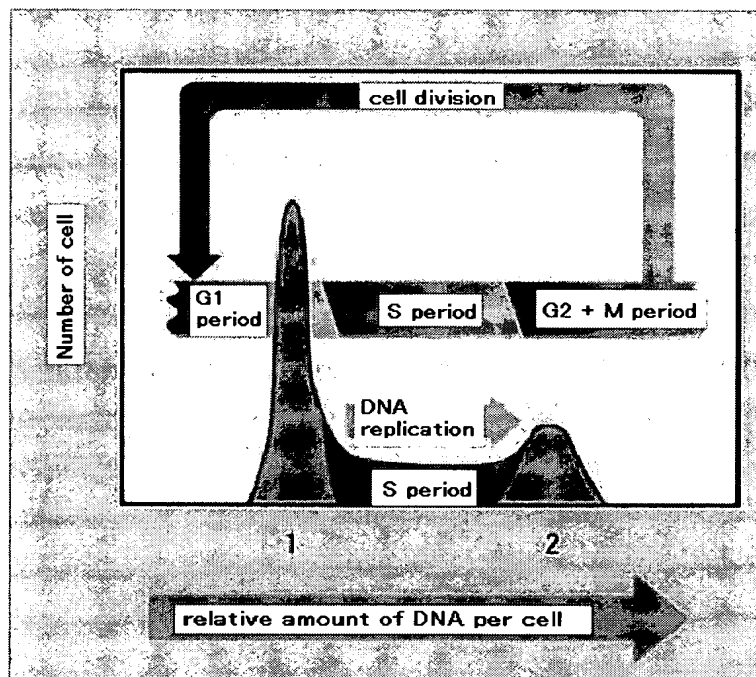
G2+M period: when a sister  
chromatid is distributed to  
daughter cells

Some anticancer drugs show a cell killing effect by exerting some influence on this cell cycle. For instance, a certain anticancer agent inhibits the S period (DNA synthesizing period) of the cell cycle. When this inhibition occurs in the cell cycle (the synthesis of DNA is inhibited), the cell cycle does not proceed well and stops in the S period.

Since the synthesis of DNA is inhibited, the cell cannot proceed to the next step (G2+M period in Fig. 1) and dies after a period of time. Material 2 shows this by using a special machine. When this machine is used, it is possible to evaluate which period the cell is in.

For details, please refer to the next page.

Fig. 2 analysis of cell cycle using FACS



Not shown in Material 2, the inhibition of the S period occurs in an early stage by adding APP. After a short time, the inhibited cell dies. Not shown in the above figure, the DNA of the inhibited cell is fragmented. That is, it becomes smaller than the DNA of an ordinary cell. Therefore, a peak appears on the left side of the G1 period in the above figure. In this actual material, this peak was observed. It is understood by checking the ratio of this peak that APP has an influence upon the cell cycle (if a peak is observed on the left side of the G1 period) and a cell killing effect.

## 2) comparison with an antioxidant

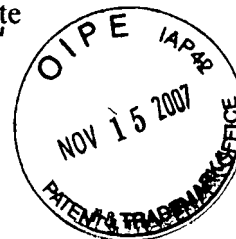
It is known that some anticancer drugs attack a cancer cell and kill it with the oxidizing substance of the drug and a radical generated by radiation in radiation therapy used in clinical care. If an antioxidant exists, it is suggested that this effect is reduced and this is reported by a large number of research papers. In this patent, APP shows an antitumor effect (cell killing effect) even though it has antioxidant activity. Two antioxidants used as controls are existent in vivo and widely used in this experimental system. Please note that these antioxidants have the same or higher antioxidant activity than APP (Material 2-B). If a certain substance has antioxidant activity, it does not show a cell killing effect due to its cell protecting effect. In fact, this effect is obtained in Material 2-A. However, APP shows a cell killing effect though it has antioxidant activity. The following two results are obtained from these.

- (1) The cell killing effect of APP is not obtained from its antioxidant activity.
- (2) APP shows an antitumor effect (cell killing effect) though it has antioxidant activity (an ordinary antioxidant reduces its anticancer function).

The above two are the main points of Material 2.



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Posted: 01/08/2003 Updated

## Antioxidants and Cancer Prevention: Fact Sheet

### Key Points

- Antioxidants protect cells from damage caused by unstable molecules known as free radicals (see Question 1&3).
- Laboratory and animal research has shown antioxidants help prevent the free radical damage that is associated with cancer. However, results from recent studies in people (clinical trials) are not consistent (see Question 2).
- Antioxidants are provided by a healthy diet that includes a variety of fruits and vegetables (see Question 4).

### 1. What are antioxidants?

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radical damage may lead to cancer. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals otherwise might cause. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, and A, and other substances.

### 2. Can antioxidants prevent cancer?

Considerable laboratory evidence from chemical, cell culture, and animal studies indicates that antioxidants may slow or possibly prevent the development of cancer. However, information from recent clinical trials is less clear. In recent years, large-scale, randomized clinical trials reached inconsistent conclusions.

### 3. What was shown in previously published large-scale clinical trials?

Five large-scale clinical trials published in the 1990s reached differing conclusions about the effect of antioxidants on cancer. The studies examined the effect of beta-carotene and other antioxidants on cancer in different patient groups. However, beta-carotene appeared to have different effects depending upon the patient population. The conclusions of each study are summarized below.

- The first large randomized trial on antioxidants and cancer risk was the Chinese Cancer Prevention Study, published in 1993. This trial investigated the effect of a combination of beta-carotene, vitamin E, and selenium on cancer in healthy Chinese men and women at high risk for gastric cancer. The study showed a combination of beta-carotene, vitamin E, and selenium significantly reduced incidence of both gastric cancer and cancer overall. (1)

- A 1994 cancer prevention study entitled the Alpha-Tocopherol (vitamin E)/Beta-Carotene Cancer Prevention Study (ATBC) demonstrated that lung cancer rates of Finnish male smokers increased significantly with beta-carotene and were not affected by vitamin E. (2)
- Another 1994 study, the Beta-Carotene and Retinol (vitamin A) Efficacy Trial (CARET), also demonstrated a possible increase in lung cancer associated with antioxidants. (3)
- The 1996 Physicians' Health Study I (PHS) found no change in cancer rates associated with beta-carotene and aspirin taken by U.S. male physicians. (4)
- The 1999 Women's Health Study (WHS) tested effects of vitamin E and beta-carotene in the prevention of cancer and cardiovascular disease among women age 45 years or older. Among apparently healthy women, there was no benefit or harm from beta-carotene supplementation. Investigation of the effect of vitamin E is ongoing. (5)

#### **4. Are antioxidants under investigation in current large-scale clinical trials?**

Three large-scale clinical trials continue to investigate the effect of antioxidants on cancer. The objective of each of these studies is described below. More information about clinical trials can be obtained using [www.cancer.gov/clinicaltrials](http://www.cancer.gov/clinicaltrials)<sup>1</sup>, [www.clinicaltrials.gov](http://www.clinicaltrials.gov), or the CRISP database at [www.nih.gov](http://www.nih.gov).

- The Women's Health Study (WHS) is currently evaluating the effect of vitamin E in the primary prevention of cancer among U.S. female health professionals age 45 and older. The WHS is expected to conclude in August 2004.
- The Selenium and Vitamin E Cancer Prevention Trial (SELECT) is taking place in the United States, Puerto Rico, and Canada. SELECT is trying to find out if taking selenium and/or vitamin E supplements can prevent prostate cancer in men age 50 or older. The SELECT trial is expected to stop recruiting patients in May 2006.
- The Physicians' Health Study II (PHS II) is a follow up to the earlier clinical trial by the same name. The study is investigating the effects of vitamin E, C, and multivitamins on prostate cancer and total cancer incidence. The PHS II is expected to conclude in August 2007.

#### **5. Will NCI continue to investigate the effect of beta-carotene on cancer?**

Given the unexpected results of ATBC and CARET, and the finding of no effect of beta-carotene in the PHS and WHS, NCI will follow the people who participated in these studies and will examine the long-term health effects of beta-carotene supplements. Post-trial follow-up has already been funded by NCI for CARET, ATBC, the Chinese Cancer Prevention Study, and the two smaller trials of skin cancer and colon polyps. Post-trial follow-up results have been published for ATBC, and as of July 2004 are in press for CARET and are in progress for the Chinese Cancer Prevention Study.

#### **6. How might antioxidants prevent cancer?**

Antioxidants neutralize free radicals as the natural by-product of normal cell processes. Free radicals are molecules with incomplete electron shells which make them more chemically reactive than those with complete electron shells. Exposure to various environmental factors, including tobacco smoke and radiation, can also lead to free radical formation. In humans, the most common form of free radicals is oxygen. When an oxygen molecule (O<sub>2</sub>) becomes electrically charged or "radicalized" it tries to steal electrons from other molecules, causing damage to the DNA and other molecules. Over time, such damage may become irreversible and lead to disease including cancer. Antioxidants are often described as

"mopping up" free radicals, meaning they neutralize the electrical charge and prevent the free radical from taking electrons from other molecules.

## 7. Which foods are rich in antioxidants?

Antioxidants are abundant in fruits and vegetables, as well as in other foods including nuts, grains and some meats, poultry and fish. The list below describes food sources of common antioxidants.

- Beta-carotene is found in many foods that are orange in color, including sweet potatoes, carrots, cantaloupe, squash, apricots, pumpkin, and mangos. Some green leafy vegetables including collard greens, spinach, and kale are also rich in beta-carotene.
- Lutein, best known for its association with healthy eyes, is abundant in green, leafy vegetables such as collard greens, spinach, and kale.
- Lycopene is a potent antioxidant found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, blood oranges, and other foods. Estimates suggest 85 percent of American dietary intake of lycopene comes from tomatoes and tomato products.
- Selenium is a mineral, not an antioxidant nutrient. However, it is a component of antioxidant enzymes. Plant foods like rice and wheat are the major dietary sources of selenium in most countries. The amount of selenium in soil, which varies by region, determines the amount of selenium in the foods grown in that soil. Animals that eat grains or plants grown in selenium-rich soil have higher levels of selenium in their muscle. In the United States, meats and bread are common sources of dietary selenium. Brazil nuts also contain large quantities of selenium.
- Vitamin A is found in three main forms: retinol (Vitamin A1), 3,4-didehydroretinol (Vitamin A2), and 3-hydroxy-retinol (Vitamin A3). Foods rich in vitamin A include liver, sweet potatoes, carrots, milk, egg yolks and mozzarella cheese.
- Vitamin C is also called ascorbic acid, and can be found in high abundance in many fruits and vegetables and is also found in cereals, beef, poultry and fish.
- Vitamin E, also known as alpha-tocopherol, is found in almonds, in many oils including wheat germ, safflower, corn and soybean oils, and also found in mangos, nuts, broccoli and other foods.

## References:

- 1) Blot WJ, Li JY, Taylor PR, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* 1993;85:1483-91.
- 2) The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effects of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 1994;330:1029-35.
- 3) Omenn GS, Goodman G, Thomquist M, et al. The beta-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. *Cancer Res* 1994;54(7 Suppl):2038s-43s.
- 4) Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular



disease. N Engl J Med 1996;334:1145-9.

5) Lee IM, Cook NR, Manson JE. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: Women's Health Study. J Natl Cancer Inst 1999;91:2102-6.

## Table of Links

1 <http://cancer.gov/clinicaltrials>